

**REMARKS**

In this Amendment, claims 3, 5, 21 and 22 are amended and claims 19 and 20 are canceled. Thus, after entry of this Amendment, claims 3, 5, 21 and 22 will be pending in this application.

Claims 3 and 5 have been amended to recite that the cross-linking agent is a vinylsulfonic acid-type cross-linking agent, as supported by the specification at, for example, the paragraph bridging pages 10 and 11 as well as the Examples. Claims 21 and 22 have been amended to be consistent with the independent claims.

No new matter has been added.

The present invention, for example as recited in claim 3, is a method for detecting a protease in a biological sample comprising the steps of: (1) contacting one of two or more substantially continuous slices of a biological sample with a dried thin membrane which comprises a protease substrate together with a cross-linking agent of a vinylsulfonic acid type, formed on a surface of a support; (2) contacting the remaining slices with a dried thin membrane which comprises a protease substrate, a cross-linking agent of a vinylsulfonic acid-type, and a protease inhibitor, formed on a surface of a support; (3) detecting traces of digestion formed on the dried thin membranes by the action of protease; and (4) comparing the trace of digestion on the dried thin membrane used in step (1) with the trace of digestion on the dried thin membrane used in step (2).

**Rejections Under 35 U.S.C. § 103(a)**

(A) At page 2 of the Office Action, the Examiner maintains the rejection of claim 3 and rejects claim 21 under 35 U.S.C. § 103(a) as obvious over Salthouse in view of Galis and Battista, substantially as set forth in the previous Office Action. Specifically, the Examiner contends the following.

The Examiner states that Salthouse teaches a method for detecting protease in a sample by contacting tissue sections with a dried thin membrane comprising collagen dried on a support, and comparing digestion of the collagen to digestion of collagen soaked in a protease inhibitor.

The Examiner states that Galis teaches a method of detecting a protease in a biological sample by contacting consecutive tissue sections with a thin membrane comprising a support holding a fluorescent substrate mixed with a protease substrate, and comparing results with a similar membrane also containing a protease inhibitor.

The Examiner states that Battista teaches a variety of cross-linking agents for collagen, and teaches that cross-linking may improve the properties of thin collagen films.

The Examiner concludes that one of ordinary skill in the art, wanting to assay for protease activity across a series of tissue sections, would have been motivated to perform the method of Salthouse using the consecutive tissue sections as taught by Galis. The Examiner further concludes that one of ordinary skill in the art wanting to improve the properties of the collagen film of Salthouse would have been motivated to add cross-linking agents to the collagen film as taught by Battista.

The Examiner asserts that one of ordinary skill in the art would have had a reasonable expectation of successfully applying the teachings of Battista to the assay of Salthouse, because Battista teaches that cross-linked collagen may be used successfully on a variety of surfaces, and for a variety of purposes.

(B) At page 4 of the Office Action, the Examiner maintains the rejection of claim 5 and rejects claim 22 under 35 U.S.C. § 103(a) as obvious over Salthouse in view of Galis and Battista as applied above, and further in view of Lawrence, substantially as set forth in the previous Office Action.

Specifically, the Examiner contends that Lawrence teaches a device for detecting proteases in samples with multiple layers.

The Examiner concludes that one of ordinary skill in the art would have been motivated to laminate a layer comprising a substrate, a cross-linking agent, and a protease inhibitor, to a layer comprising a substrate and cross-linking agent, in order to measure protease activity with the method of Salthouse in a single sample with a single test element.

The Examiner further concludes that one of skill in the art would have had a reasonable expectation of successfully applying the teachings of the cited art to perform the claimed method because Battista teaches that cross-linked collagen may successfully be used in multi-layered elements and Lawrence teaches that multi-layered elements may be used to detect proteases.

(C) At page 9 of the Office Action, the Examiner rejects claims 19 and 20 under 35 U.S.C. § 103(a) as obvious over Salthouse in view of Galis and Battista, as applied to claims 3 and 21, and further in view of U.S. Patent 5,219,992 to Specht.

Specifically, the Examiner states that Specht teaches thin films of gelatin or collagen containing vinylsulfonyl cross-linkers, and that such may be used in multi-layered elements.

The Examiner concludes that, in the absence of evidence of unexpected results, it would have been obvious to one of ordinary skill in the art to have used a vinylsulfonyl crosslinking agent as taught by Specht, since Specht teaches that vinylsulfonyl crosslinking agents are suitable for collagen layers.

### **Response to Examiner's Rejections**

Claims 19-20 have been canceled, rendering the rejection of these claims moot.

Applicants have amended claims 3 and 5 to recite that the cross-linking agent is a vinylsulfonic acid-type cross-linking agent. Since the Examiner admits that none of Salthouse, Battista, Galis or Lawrence teaches a vinylsulfonic acid-type cross-linking agent, and since claims 19 and 20 are not rejected as obvious over the combination of Salthouse, Battista, Galis and Lawrence, the rejections of claims 3 and 5 and the rejections of dependent claims 21 and 22 over the combination of Salthouse, Battista, Galis and Lawrence should be withdrawn.

In response to the Examiner's rejection in view of Specht, Applicants assert that the cross-linking agents of the present invention provide an unexpected advantage over the prior art.

Application No. 09/917,897  
Amendment under 37 CFR § 1.116

The use of the vinylsulfonic acid-type cross-linking agent in the method of the present invention allows for better control of sensitivity and better reproducibility, as described at page 8, lines 11-17 of the specification.

For example, sample Nos. 116, 117, 118, and 127 of Table 1 (pages 21 and 22 of the specification), which are all 7 µm thick, were prepared so as to contain a different amount of the cross-linking agent 1,2-Bis (vinylsulfonyl-acetoamido) ethane. The experimental results obtained with these samples show that a higher amount of the cross-linking agent reduces the degree of digestion by a protease (see Table 5 at page 29). The higher amount of cross-linking agent in sample 118 reduces protease digestion over the lesser amounts contained in samples 116 and 117 (as measured by optical density and membrane thickness).

These results are unexpected over the teachings of the cited art, and as such, Applicants request reconsideration and withdrawal of this rejection.

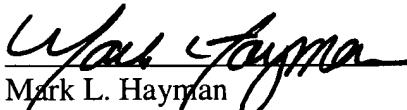
### Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Application No. 09/917,897  
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The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

  
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